

**FY14 Application for Nursery Research Funding**  
**Washington State Department of Agriculture - Nursery License Surcharge**  
(Please use one application packet including the Progress Report page for each proposal.  
You must use our form - failure to do so may result in not funding your project.)

14-002

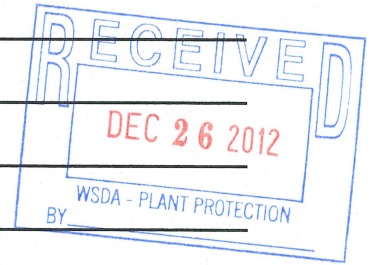
Project Title: Use of advanced sequencing to facilitate the introduction of virus-tested fruit trees

Project Leader: Ken Eastwell

Institution (if any): Washington State University, Clean Plant Center – Northwest

Mailing Address: 24106 North Bunn Road, Prosser, WA 99350

Email: keastwell@wsu.edu



Project Phone Number: ( 509 ) 786-9385 Cellular/Pager Number: ( 509 ) 781-1445

**Note: Project leader or his/her designee must be available at above project phone number on March 1, 2013 between the hours of 10:00-12:00 and 1:00-3:00.**

Amount Requested for (FY13) July 1, 2013 to June 30, 2014: \$26,957

Start Date: July 1, 2013

Completion Date: June 30, 2016

(Check One) New Project ☒

Continuing ☐

If this is a multiple year project, please estimate and list the following information for each future July 1 - June 30 period listed below through project completion:

Fiscal Years (FY)	July 1, 2013 to June 30, 2014	July 1, 2014 to June 30, 2015	July 1, 2015 to June 30, 2016	July 1, 2017 to June 30, 2018	July 1, 2018 to June 30, 2019
\$ Amount Needed	\$26,957	\$29,635	\$33,340		

If you are increasing the above amounts since your last application, please explain why:

\*Please list all other sources and amounts of funding for this project for the current year only: (Please notify us by March 1st if other funding has been approved and from where.)

Source	\$ Amount Applied For	Approved	Pending Date of Notification
USDA-APHIS	\$68,000	\$68,000	

Total Amount Needed to Fund Project (Include all sources) \$ 94,957

If total amount from all sources is not granted, will you be able to complete the project? No

Explain:

The objective of this project is to compare current standard virus testing procedures with the speed and accuracy of next generation sequencing. USDA-APHIS funds partially offset the cost of testing fruit tree selection by current protocols. Funding from Nursery Research Funding is requested for next generation sequencing of a representative subset of these selections. Both testing protocols are necessary for the comparison to be valid.

**Submit 16 copies of this proposal to: Tom Wessels, Plant Services Program Manager, P.O. Box 42560, Olympia, WA 98504-2560, twessels@agr.wa.gov, or fax (360) 902-2094. All applications must be postmarked by December 31, 2012.**

**Please summarize the purpose of this research: (you may attach additional sheets if necessary or submit this summary in your own format)**

Currently, the introduction of new fruit tree cultivars from foreign countries requires a very time consuming and expensive virus testing protocol based on herbaceous indexing, woody greenhouse indexing, woody field indexing, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISA). The cost of this complex testing protocol ranges from \$3,467 to \$3,673 for each pome fruit or stone fruit tree, respectively, that is brought into the U.S. The virus testing protocol requires a minimum of three years to complete. In the last few years, the cost of next generation sequencing has decreased dramatically and it has become more readily accessible. Once exclusively in the domain of megasequencing projects such as the human genome project, next generation sequencing technology can now be performed for one-half the cost of the full complement of virus tests, and the results are available within weeks. However, the international movement of vegetatively propagated perennial plant material is heavily regulated. In order to revise regulations to embrace this new technology, sufficient data must be obtained to demonstrate the utility of next generation sequencing in comparison to current virus detection methods. This project seeks to evaluate the effectiveness of contemporary technology for detection of viruses in fruit trees to increase the accessibility of U.S. nurseries to new fruit tree cultivars. Adoption of this technology would accelerate the delivery of virus-tested fruit tree cultivars to the industry without compromising efficacy of testing.

**Methods of research:**

Selections received by the program will be subjected to the full complement of virus testing procedures as specified in standard operating procedures. Of the 70 selections introduced with funding from USDA-APHIS, 15 will be selected for next generation sequencing to represent foreign and domestic introductions, and include samples from the genera *Malus*, *Prunus* and *Pyrus*. Dormant budwood is received and budded onto virus-tested rootstock. Young fully expanded leaves from emerging shoots will be collected, from which total RNA is extracted. A copy DNA library will be prepared from the RNA, and labeled. The labeled samples will be subjected to multiplexed 50 bp single-end Illumina sequencing. This process typically yields in excess of 15 million short sequences per sample. These sequences will be assembled by computer and the resulting segments analyzed for the presence of signature sequences indicative of the presence of plant viruses.

Over the three-year course of this project, data will be collected and summarized from each year. If the results support the adoption of next generation sequencing as a standard diagnostic tool, this database will be the foundation of a request to USDA-APHIS to permit the use next generation sequencing as a replacement for the complex testing procedures currently used. It is predicted that the speed and accuracy of virus testing by this method would provide heightened security to the fruit tree industry, and more rapid access to newly developed fruit tree cultivars. New cultivars are most profitable in the first years after they are produced, so the speed is important for producers. Unique viruses not recognized by existing protocols could also be identified. This has been well documented in grapevines. In fruit trees, a similar example is the detection of *Cherry virus A* that was only initially revealed through sequencing efforts.

**Expenditure Breakdown:**

**(Please include salaries, supplies, travel, etc.)**

Salaries (0.30 FTE Post Doctoral researcher)	\$11,858
Benefits (State standard rate = 43%)	5,099
Supplies for analyzing 15 samples:	10,000
. materials for isolating RNA from plants	
. enzymes for cDNA labeling	
. Illumina sequencing	
. computer time purchase for sequence analysis	
Travel	0
Overhead	0
<b>TOTAL REQUEST</b>	<b>\$26,957</b>

**The information requested on this page will have a direct bearing on whether your research request is approved or denied. Letters of support by the industry are also encouraged.**

**Note: Funding is not available for general overhead cost.**